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Studies on Structural Lipids in the Stratum Corneum

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To determine the mode of existence of structural lipids in the stratum corneum, lipids induced in the thick horny cell layers of guinea pig skin by n-hexadecane treatment were analyzed.

Total lipids in the stratum corneum were extracted by Folch's solution and the ratio of phospholipids to free cholesterol was 1:8. Lipids extracted by non-ionic surfactant treatment appeared to originate from both the cell surface and intercellular space of the stratum corneum and the ratio of free cholesterol to phospholipids in this lipid fraction was much higher than that in the total lipids of the stratum corneum extracted by Folch's solution.

(Key Words: Stratum Corneum, Non-ionic Surfactant, Free Cholesterol, Phospholipids)

INTRODUCTION

The outermost layer of the epidermis is composed of horny cells which are the final products of the keratinization process of epidermal cells. The horny cell, differentiated from the basal cell via the spinous and granular cells, is a flat cell and has no nucleus. This cell consists of a thick cell membrane, keratin fibers and an interfilamentous substance derived from keratohyalin granules and the residue of intracellular organellae (7). The major component of the horny cell is the protein keratin. However, it has been suggested that lipid components are present in the structure of horny cells in accordance with the fluid mosaic model of cell membranes proposed by Singer and Nicolson (18).

It is well known that large amounts of lipids exist on the skin surface, and many investigations were performed concerning the role of this skin surface lipid (14). Most of this lipid is excreted into the skin surface from sebaceous glands (16), although small amounts of this lipid originate from the epidermal tissue. The major components of sebaceous lipids measured in an isolated sebaceous gland were triglycerides, squalene and wax esters (9). On the other hand, epidermal lipids consisted mainly of cholesterol (free and esterified) and phospholipids (16). Triglycerides were also synthesized in epidermal cells (14, 15), but the amounts appear to be negligible compared with the amounts of sebaceous gland lipids in the skin surface lipids.

To dermine the mode of existence of structural lipids in the stratum corneum, the amounts of cholesterol and phosphlipids in the stratum corneum were measured. Lipid samples were obtained from hyperkeratotic guinea pig epidermis by extraction with various solvents. At the same time,

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morphological changes in the stratum corneum caused by such treatment were observed by electron microscopy.

MATERIALS AND METHODS

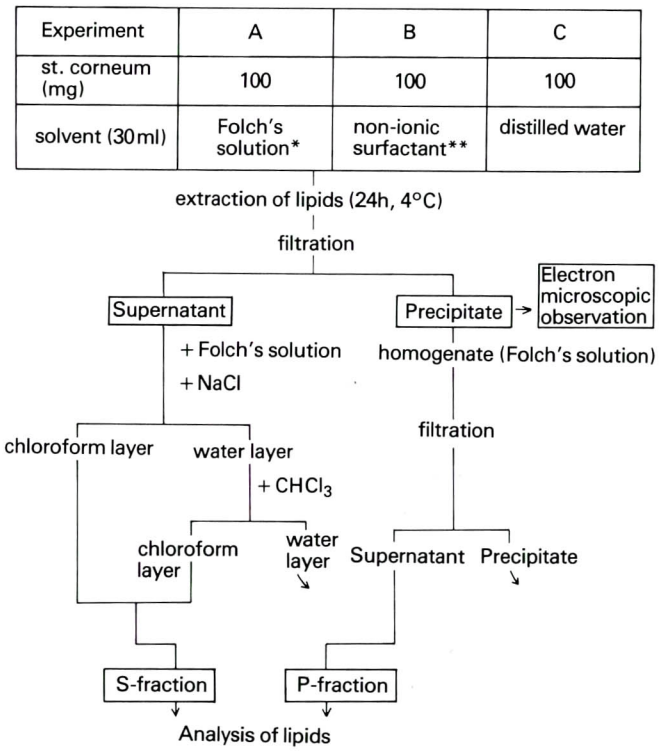
Production of hyperkeratotic epidermis

Hyperkeratotic epidermis was produced by n-hexadecane treatment on the backs of guinea pigs (10), and the thick stratum corneum (horny cell layers) was collected by tweezers.

Extraction of lipids in the stratum corneum

The stratum corneum was cut into small pieces (3 × 2 mm) by scissors and lipids were extracted by the methods described in Fig. 1.

Distilled water, a non-ionic surfactant and Folch's solution (2) were used for the extraction of lipid samples (S-fraction). After the extraction of lipids from the stratum corneum with these solvents, the materials were homogenized with a Potter-Elvehjem glass homogenizer and total lipids were extracted by Folch's solution (P-fraction).



* chloroform/methanol = 2/1
** polyoxyethlene alkyl ether ($R = C_{11}$ 5%, C_{12} 43%, C_{13} 45%, C_{14} 6%, $\bar{p} = 8$) 0.5% aqueous solution.

Fig. 1 Flow diagram of the extraction of lipids in the stratum corneum

Analysis of lipids

Quantitative analysis of cholesterol was performed by gas-liquid chromatography using n-tetratriacontan as the internal standard (1).

Phosphipids were analyzed by Long's method (12).

Electron microscopic observations

Treated specimens of the stratum corneum were fixed with glutaraldehyde and postfixed with osmium tetraoxide, and then embedded in Epon 812. Ultrathin sections were prepared and were doubly stained with uranyl acetate and lead citrate.

RESULTS

The amount of free cholesterol and phospholipids in the S and P fractions are shown in Tables 1 and 2, respectively. The amounts are expressed as μg per mg of dry tissue.

In the S-fraction, lipids could not be extracted by distilled water. Therefore, the lipids extracted with Folch's solution from this residue, shown in Experiment C on the P-fraction (Table 2), are the total lipids in this stratum corneum. Total phospholipids and free cholesterol contents in this horny cell layer were 0.84μg and 6.83μg per mg of dry tissue, respectively. The weight ratio of phospholipids to free cholesterol in total lipids in the stratum corneum was 1:8.

In the S-fraction, Folch's solution extracted most of the structural lipids (Table 1), while the non-ionic surfactant extracted about half of the phospholipids and free cholesterol among the total lipids in the stratum corneum (Table 1).

Electron microscopic observations of the stratum corneum after lipid extraction with these solvents showed some morphological changes. Treatment with distilled water had no influence on the fine structure of horny cells including the cell membrane, keratin filament, desmosomes and intercellular space (Fig. 2). Non-ionic surfactant treatment caused the mutual separation of horny cells and the loss of desmosomes, but some amorphous substances still adhered to the cell surface (Fig. 3). Treatment with Folch's solution showed some morphological changes i.e. a high density of cell membrane and intracellular substances, and the reduction of cell size (Fig. 4).

Table 1 Lipid values in the S-fraction

Experiment	No.	Lipids (μg/mg of dry tissue)	
		phospholipids	free cholesterol
A	1	0.57	6.45
	2	0.79	6.15
	3	0.55	6.30
average		0.64	6.30
B	1	0.26	3.75
	2	0.31	3.60
	3	0.17	3.00
average		0.25	3.45
C	1	0	0
	2	0	0
average		0	0

Table 2 Lipid values in the P-fraction

Experiment	No.	Lipids (μg/mg of dry tissue)	
		phospholipids	free cholesterol
A	1	0.21	0.60
	2	0.21	0.60
	3	0.31	0.30
average		0.24	0.50
B	1	0.42	3.54
	2	0.34	3.53
	3	0.55	3.53
average		0.44	3.53
C	1	0.85	6.75
	2	0.83	6.90
average		0.84	6.83

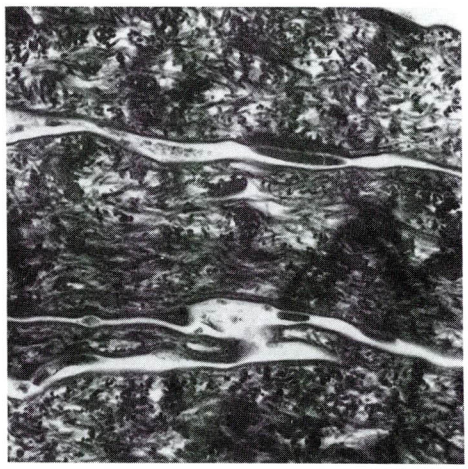


Fig. 2 Electron micrograph of horny cells treated by distilled water (10,000×3.4)

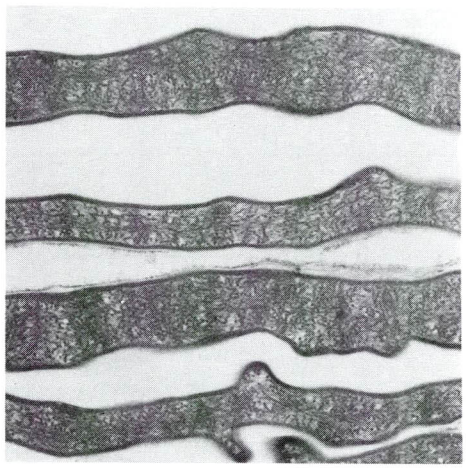


Fig. 3 Electron Micrograph of horny cells treated by a non-ionic surfactant (10,000×3.4)

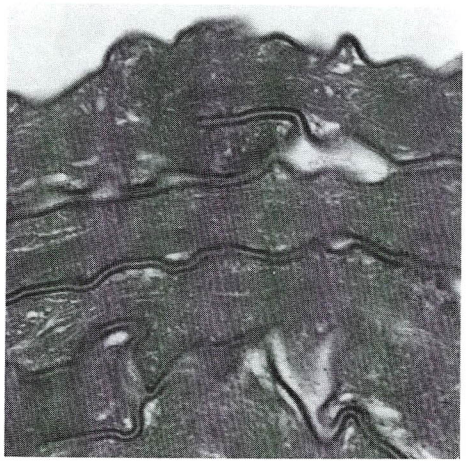


Fig. 4 Electron micrograph of horny cells treated by Folch's solution (10,000 × 3.4)

DISCUSSION

The total cholesterol and phospholipid content in horny, spinous and basal cell layers of human palm epidermis which lack sebaceous glands was measured by Kooyman in 1932 (11). The correlation between these lipids and the differentiation of these cell layers form basal cells to horny cells was observed. The ratios of phospholipids to cholesterol were 1.8, 0.7 and 0.2 in basal, spinous and horny cell layers, respectively and the total cholesterol value was five times higher than the phospholipid value in the horny cell layer. In 1975, Gray and Yardley (5) separated pig epidermis into basal and spinous, granular and stratum corneum cells, and measured the lipid compositions of these cells. Phospholipids were predominant in the basal and spinous cells, but the percentage of phospholipids in the total lipids in the stratum corneum was less than 0.1%. The stratum corneum cells contained more cholesterol (23% of total lipids) than either the granular cells (18%) or the basal and spinous cells (8%) (5).

The following two possibilities are suggested concerning the existence of lipids in the stratum corneum: (i) presence in the intercellular space of horny cells or adhesion to the cell surface, and (ii) presence in the cell membrane of horny cells or in the horny cells themselves. The origin of lipids in the intercellular space of the cell surface of horny cells has been suggested as (a) intercellular substances derived from serum in living cell layers, (b) lipids derived from sebaceous glands and (c) lipids derived from epidermal cells, especially in granular cells. The lamellar granules, one of the intracellular organellae appearing in the granular cell layer and also known as Odland's bodies, keratinosomes or cementosomes, had a myelin structure and were suggested to contain phospholipids (19). The contents of this granule, referred to as a cementing substance, were excreted into the intercellular space and were digested by phospholipase C (6). These relationships are illustrated in Fig. 5.

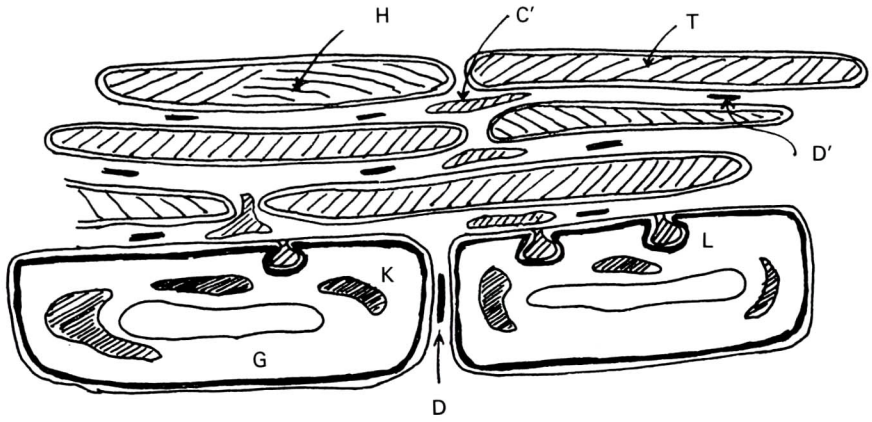


Fig. 5 Illustration of the ultrastructure of horny and granular cell layers of the epidermis
H: Horny cell, G: Granular cell, T: Keratin fiber, K: Keratohyalin granule, D: Desmosome, L: Lamellar granule, D': Residue of desmosome, C': Degradation product of cementosome

In this study, phospholipids and free cholesterol in lipids of the stratum corneum were measured and the modes of existence of these lipids in the stratum corneum were suggested to be (i-a), (i-c) or (ii) above.

Phospholipids are considered to act on the adhesion mechanism of cells in the mouse liver (4). Loss of the intercellular gap junction in the mouse liver with 60% acetone treatment was observed by electron microscopy, and phospholipids were found in the extracted solvent (4), but tight bonds still remained after this treatment (4).

In this experiment, a non-ionic surfactants extracted lipids from the stratum corneum and separated horny cells from each other. Non-ionic surfactants are considered to remove the surface protein which adheres to the cell membrane by weak hydrophobic binding, but not to remove the structural protein and also not to destroy the structure of the cell membrane (8). In the extraction of lipids in the stratum corneum, non-ionic surfactants were milder than Folch's solution and it is suggested that most of the intercellular and cell surface lipids, instead of cell membrane or intracellular lipids, were removed by non-ionic surfactant treatment. Extraction of lipids by Folch's solution showed an increase in the electron density of the cell membrane and intracellular components. These results suggest that Folch's solution extracts both intercellular and cellular lipids.

In conclusion, the weight ratio of phospholipids to free cholesterol among the whole lipids of the stratum corneum was 1:8. After removal of intercellular and cell surface lipids by non-ionic surfactant treatment, the ratio of phospholipids to free cholesterol in the horny cell lipids was also about 1:8 (Experiment B in Table 2). From these data, we estimated the constitution of horny cell membrane lipids because of the difficulty of obtaining pure materials. If this ratio expresses the value of horny cell membrane lipids, it is very high compared with that of other organs. For example, the molecular ratio of cholesterol to phospholipids in animal or human cell membrane lipids ranged between 0.2 and 2.4 (3). The flexibility

of the cell membrane was considered to be decreased in proportion to the increase of cholesterol content in the liquid mosaic model of the lipid bilayer (17). A high free cholesterol content in the stratum corneum of the epidermis, the outermost layer of the skin, may be reasonable considering its function.

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